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2503-1170

IN THE U.S. PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of	Appeal No.
Daniele CALISTRI et al.	Conf. 1643
Application No. 10/547,669	Group 1637
Filed September 2, 2005	Examiner M. Staples

METHOD FOR THE IDENTIFICATION OF COLORECTAL TUMORS

APPEAL BRIEF

MAY IT PLEASE YOUR HONORS:

(i) Real Party in Interest

The real party in interest in this appeal is the assignee, INSTITUTO ONCOLOGICO ROMAGNOLO COOPERATIVA SOCIALE A R.L., of Forli, Italy.

(ii) Related Appeals and Interferences

Neither the appellant, appellant's legal representative nor does the assignee know of any other prior or pending appeals, interferences or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(iii) Status of Claims

Claims 1, 9, 10, and 12 remain in the application.

Claim 12 was withdrawn from further consideration for being directed to non-elected subject matter.

This appeal is taken from the final rejection of claims 1, 9 and 10 in the Office Action of August 30, 2010 ("Official Action").

Claims 2-8, 11 and 13-22 were cancelled.

(iv) Status of Amendments

The claims were last amended in the Amendment filed February 16, 2010, which are set forth in the Claims Appendix.

(v) Summary of the Claimed Subject Matter

The claimed subject matter, as defined in the sole independent claim 1, is directed to a method for determining the presence of colorectal tumors in a human subject, which comprises:

a) extracting DNA from stool samples;

(Specification Page 2, lines 21-25)

b) amplifying by PCR:

i) p53 gene fragments corresponding to exons 5-8 using the following pairs of primers labeled with fluorescent markers:

1)ctcttcctgcagtactcccctgc(SEQ ID NO:1) and gccccagctgctcaccatcgcta (SEQ ID NO:2),

2)gattgctcttaggtctggcccctc (SEQ ID NO: 3) and ggccactgacaaccacccttaacc(SEQ ID NO: 4),

3)gcgttgtctcctaggttggctctg (SEQ ID NO: 5) and caagtggctcctgacctggagtc (SEQ ID NO: 6),

4)acctgatttccttactgcctctggc(SEQ ID NO: 7) and gtcctgcttgcttacctcgcttagt (SEQ ID NO: 8), and

ii) APC gene fragments using the following pairs of primers labeled with fluorescent markers:

1)aactaccatccagcaacaga (SEQ ID NO: 9) and taatttggcataaggcatag (SEQ ID NO: 10),

2)cagttgaactctggaaggca (SEQ ID NO: 11) and tgacacaaagactggcttac (SEQ ID NO: 12),

3)gatgtaatcagacgacacag(SEQ ID NO: 13) and ggcaatcgaacgactctcaa(SEQ ID NO: 14),

4)cagtgatcttccagatagcc(SEQ ID NO: 15) and aaatggctcatcgaggctca(SEQ ID NO: 16);

(Specification Page 2, line 26 to Page 3, line 1 in light of the identification of p53 gene fragments and APC gene fragments on Page 7)

c) quantifying the amplified fragments and identifying the amplified fragments as amplicons;

(Specification Page 3, line 2)

d) calculating the total amount of different amplicons;

(Specification Page 3, line 3)

and

e) comparing the the total amount of amplicons with a reference value determined on the basis of case series comprising healthy subjects and patients affected by colorectal tumors, wherein a total amount of amplicons higher than the reference value is indicative of the presence of colorectal tumors in said human subject.

(Specification page 3, line 4 in light of the Results described on page 8, line 23 to page 9, line 5)

(vi) Grounds of Rejection to be Reviewed on Appeal

a. Whether claims 1, 9 and 10 would have been obvious within the meaning of 35 U.S.C. §103(a) over SHUBER WO 2001/042502(“SHUBER”), KMIEC et al. WO 2001/73002 (“KMIEC”), ALBERTSEN et al. US 6,114,124 (“ALBERTSEN”), and BUCK et al. 1999(“BUCK”).

b. Whether claims 1, 9 and 10 would have been obvious within the meaning of 35 U.S.C. §103(a) over SHUBER,

KMIEC, ALBERTSEN and BUCK, as prompted by the IDS submission of July 12, 2010.

(vii) Arguments

a. None of claims 1, 9 and 10 is obvious over SHUBER, KMIEC, ALBERTSEN and BUCK.

SCHUBER was offered teaching the claimed method, except for the claimed pairs of primer sequences.

KMIEC was offered for teaching sequences comprising SEQ ID NOs 10 and 16 and sequences comprising the sequences of primer pairs SEQ ID NOs 13 and 14, and ALBERTSON was offered for teaching a sequence comprising SEQ ID NO: 15. However, neither one of these documents teaches the claimed any of the claimed primers *per se* according to SEQ ID NOs 9, 10, 11, 12, 13, 14, 15 and 16.

BUCK, allegedly, "expressly provides evidence of the equivalence of primers". See, e.g., the Action of April 16, 2009, page 11, first full paragraph, to which the Official Action refers.

There does not appear to be an appreciation of the teachings of SHUBER, KMIEC, ALBERTSEN and BUCK as understood by one of ordinary skill in the art, especially as compared to the claimed features. Indeed, their combination fails to teach or suggest the claimed invention. The teachings of the references,

as well as the alleged teachings of their combination are discussed below.

SHUBER

The analysis method used by SHUBER, which is based on agarose gel electrophoresis (e.g., as described on page 18, line 18 and the Examples of SHUBER), differs from the claimed invention and, thus, cannot give the same results as those obtained by the claimed invention. That is, the sole difference is not simply the primers as stated in the rejection.

This is confirmed by the Neoplasia article, originally cited in the amendment filed February 15, 2010, which is provided in the Evidence Appendix.

This article is based on a study by the Appellant. The Appellant demonstrated that a method based on agarose gel electrophoresis (as used by SHUBER), which is a "L-DNA analysis", and a method based on fluorescence quantization (i.e., using fluorescent labeled primers as claimed), which is a "FL-DNA" (Fluorescence long DNA) analysis, yield different results in terms of accuracy in identifying neoplastic lesions. (See, e.g., the Abstract in light of description of the methods L-DNA Analysis and FL-DNA Analysison Page 537.) The study demonstrated that the fluorescent method as opposed to the agarose gel electrophoresis method improves sensitivity by 26%. (See, e.g., the abstract of the article, which compares 50% sensitivity of

DNA amplification to 76% sensitivity of fluorescence DNA determination.)

The Examiner stated that the claimed primers are not specifically taught by this article, and, thus, the article fails to support that the primers are novel. However, this article was not submitted to establish novelty of the primers themselves. Instead, the article was submitted to demonstrate that one of ordinary skill in the art would have not have looked to SHUBER, who uses an agarose gel electrophoresis method, which is known to be less sensitivity than a fluorescent method, as a starting point to approach the claimed invention.

This difference between an agarose gel electrophoresis method and a fluorescent method is not apparent from SHUBER, nor was it considered in SHUBER.

The dynamic range of the two systems is an important characteristic needed in order to obtain good discrimination between cancer patients and healthy individuals. In fact, even when the same approach is used (e.g., agarose gel electrophoresis as utilized by SHUBER), different DNA markers can give different results in terms of dynamic range and of the possibility of distinguishing between samples with similar but not identical levels of long DNA (L-DNA) molecules.

Indeed, the ethidium bromide used by SHUBER (page 20, line 31) is a not a very effective reagent for this type of analysis as generally known in the prior art. For example, in

Gerard et al. Cancer Research 58:3957-64, 1998(cited in the IDS of July 12, 2010), ethidium bromide-stained agarose gel is described as producing false positives in quantitative PCR assays (see, e.g., the paragraph at the bottom of page 3962 bridging the two columns). Similarly, this lack of sensitivity of ethidium bromide in PCR methods is disclosed in Schneeberger, et al., Methods and Applications 4:234-238, 1995(cited in the IDS of July 12, 2010). See, e.g., the columns between Figures 2 and 3 on page 35.

The Examiner disregarded these documents because the claims recite "comprising", and, thus, do not exclude ethidium bromide. However, these documents further distance SHUBER from the claimed invention by showing that SHUBER chose a reagent, as well as a method, with a lack of sensitivity.

As the ability to discriminate between very similar quantities is fundamental in order to correctly identify patients with neoplastic colorectal disease (as evidenced by the Neoplasia article), the claimed method significantly adds to the accuracy already obtained by SHUBER. This is a fact that SHUBER did not take into consideration, erroneously stating that various methodological approaches may determine equivalent results. See, e.g., in the paragraph bridging pages 18 and 19 of SHUBER.

Thus, SHUBER fails to teach or suggest the necessary steps to approach the claimed invention.

KMIEC and ALBERTSEN

In support of the Examiner's position that the claimed primers are "simply represent structural homologs" of KMIEC and ALBERTSEN, the Examiner cited to *In re Deuel*, 34 USPQ 2d 1210 (Fed Cir. 1995). See, e.g., on page 10 of the Office Action from April 16, 2009, from which this ground of rejection is based.

However, the Examiner's reliance on *In re Deuel* is improper. Neither KMIEC nor ALBERTSEN would have led one skilled in the art to the particular primer or would have indicated that that any of the primers should be prepared. There is no teaching of a specific, structurally-definable compound in accordance with the primers recited in the claims or a suggestion of the specific molecular modifications necessary to achieve the claimed invention. Accordingly, KMIEC and ALBERTSEN fail to offer any guidance to the select the claimed primers for use in the method outlined in SHUBER.

BUCK

BUCK was offered as further evidence of the equivalency of primers, i.e., "every primer would have a reasonable expectation of success" as stated at the bottom of page 11 of the Office Action from April 16, 2009.

However, BUCK fails to remedy the shortcomings of SHUBER, KMIEC and ALBERTSEN for at least two reasons.

First, the "equivalence" of the primers from the same DNA region suggested by BUCK is not relevant to either SHUBER or the claimed invention.

That is, BUCK suggests equivalence among primers in terms of their use for the sequence analysis, i.e., characterization or qualification, of the same DNA regions. See, e.g., the Abstract of BUCK.

However, the claimed method concerns the quantification of genomic DNA molecules/fragments (e.g., in step (c) of claim 1). This is different from the qualification of genomic DNA through sequencing according to BUCK because the performance and correspondence required for a sequence analysis is different from those needed for a quantification study.

For example, in sequencing analysis, only a qualitative characterization (sequence reading for gene discovery, mutation analysis, SNPs etc.) can be made. The main factor to be considered is the specificity of the PCR amplicon and purity in order to obtain a good sequencing result. In order to perform DNA quantity evaluation, however, in addition to having a good PCR amplicon quality, it is also important to have a good linearity of the amplification curve and a good correlation with cycle numbers and cycle temperature.

Thus, reading the genomic DNA sequence for qualification is not directly correlated with that of quantifying the DNA molecules present in the samples or of determining their

integrity. Indeed, in the claimed invention, the quantity of amplified DNA for each analyzed region is required, and it is the quantity of non fragmented DNA amplified by the claimed method, not the sequencing analysis, which determines the results and makes it possible to identify the neoplastic lesions.

Second, BUCK requires high quality genomic DNA extracted from specimens for which the quality and quantity of DNA recovery is generally good or very good.

This is a substantial difference between BUCK and the claimed invention. The genomic DNA as claimed is extracted from stool, which is generally poor in both quality and quantity, as evidenced by Loktionov in the Evidence Appendix, which was initially cited the amendment of February 16, 2010. See, e.g., the paragraph bridging pages 2282 and 2283.

The Examiner did not appreciate that the quality of DNA is relevant in that it influences the results and also the possibility of obtaining similar results using different amplification conditions, e.g. different primers.

Thus, it is impossible to make a direct comparison with the primers used by BUCK, especially in view of the fact that BUCK teaches away from a stool sample as the source of DNA as suggested by SHUBER and as claimed.

The Combination

According to the combination as proposed by the Examiner, one should expect every primer to provide a successful result for colorectal cancer detection.

The Appellant had provided experimental data using different primers that have been designed with the same genomic regions in order to demonstrate that these primers do not produce comparable results in terms of specificity of colorectal cancer detection.

Two Declarations under Rule 132 were filed to demonstrate this fact: the first filed March 14, 2008, and the second filed February 16, 2010.

Both declarations include Table 1 shows the results, in terms of FL-DNA (Fluorescence long DNA) values expressed as nanograms, obtained by two different approaches:

i) the primers as recited in the claims, and

ii) a new series of primers designed with similar genomic regions, i.e., “new primers”.

In the first declaration, Tables 2a/b showed the sensitivity and specificity of the two different approaches. In Table 2a, using the primers recited in the claimed invention, it is possible to obtain good sensitivity and specificity with different cut-offs (see, for example, 10-15 or 20 ng cut-offs). Conversely, using the “new primers” (Table 2b), sensitivity and specificity suffers, and, as a result, these “new primers” do not

allow one to identify an accurate (high sensitivity and specificity) cut-off for colorectal cancer detection.

The second declaration was filed to more closely compare the sensitivity and specificity of the "new" primers and claimed primers in a table 2. Additionally, a sample concordance correlation coefficient (pc) is 0.0456 was provided which confirms that different primers had indeed produced the different values.

One conclusion from the results which can be made is that the two sets of primers, albeit located in close regions of the target sequences, determine significantly different results in terms of FL-DNA, as evidenced by the sample concordance correlation coefficient pc=0.0456.

Another conclusion from the results is that the different quantitative PCR readouts observed with the two sets of primers determine unexpected results in terms of accuracy to detect colorectal cancer. Table 2 shows that at the same cut-off, the differences in terms of sensitivity and specificity are relevant and unexpected. The specificity still remains over 80% for the claimed primers, only 10-20% lower than the specificity obtained by new primers, whereas the sensitivity for the claimed primers was 50% or higher compared to that of the new primers which fell to 10% at the 20, 25 and 30 ng cut-offs.

The Examiner challenged the results by alleging that the differences might have resulted from error or measurement.

However, as viewed in Table 2 (or as presented in tables 2a and 2b of the first declaration), there is a clear trend in the sensitivity and specificity relative to the cut-offs for each primer, which strongly suggests that these differences are not merely the result of random errors.

As the purpose of this test is to detect colorectal cancer patients, this higher sensitivity is more important than specificity if one considers that false positive results would make necessary further diagnostic tests as the only consequence, whereas false negative results may have serious, negative consequences for the patient health.

Thus, while the "claimed" and "new" primers reported in the Declaration may well give comparable PCR amplification efficiencies, they are not equivalent when compared for their ability to discriminate among colorectal tumor patients and non-tumor individuals using a quantitative PCR-based method of diagnosis of colorectal tumors as in the claimed invention.

This selection of primers that allows for a better detection of colorectal tumor patients is not suggested by SHUBER, BUCK, KMIEC or ALBERTSEN.

These results are unexpected in that they are contrary to the teachings of the combination as interpreted by the Examiner: “every primer would have a reasonable expectation of success” for a method for determining the presence of colorectal tumors in a human subject. That is, there is no direct

correlation between the sensitivity of an amplification technique and the efficacy of a screening method.

For example, it is possible that a more accurate or more sensitive amplification method results in a substantially identical or even worse capability of differentiating between affected and unaffected individuals. The two aspects must be kept separate, because a more sensitive detection of amplicons may only show a higher level of DNA without discriminating between the two groups of individuals.

Therefore, the rejection of claims 1, 9 and 10 should be reversed.

b. None of claims 1, 9 and 10 is obvious over SHUBER, KMIEC, ALBERTSEN and BUCK, as prompted by the IDS submission of July 12, 2010.

This rejection was prompted by submission of the IDS which included the Neoplasia article and Loktionov, which were previously provided as evidentiary documents in the appendix of a replay, and the Gerard et al. and Schneeberger et al. documents, which were mentioned as evidence of the notoriously well known failings of ethidium bromide for the use in PCR assays.

SHUBER, KMIEC, ALBERTSEN and BUCK were offered for the same reasons as those presented with respect to ground of rejection **a**, as discussed above.

The Examiner further concluded that SHUBER teaches "the optimization of amplification through selection of the primer sequences, placement of primer sequences and other factors affecting results for cancer detection was known in the prior art" based on the following:

> "Each of the methods described above are based upon the principle that an intact nucleic acid, or a segment of an intact nucleic acid, in a sample is diagnostic. Thus, variations on the methods described above are contemplated. Such variations include the **placement of primers**, the number of primers used, the target sequence, the **method for identifying sequences, and others**. For example, in the method depicted in Figure 13, and described above, it is not necessary that the numbers of forward and reverse primers be equal. A forward primer may, for example, be used to amplify fragments between two reverse primers. **Other variations in primer pair placement** are within the skill in the art, as are details of the amplification reactions to be conducted. Finally, as represented in Figures 12 and 13, capture probes may be used in methods of the invention in order to isolate a chosen target sequence" (emphasis by Examiner, see the 1st paragraph on p. 17).

See, e.g., page 19, second full paragraph of the Official Action.

However, this additional commentary by SHUBER does not overcome the shortcomings of any of SHUBER, KMIEC, ALBERTSEN and BUCK or their combination. While this commentary mentions variations including the placement of primers and method for identifying sequences, there is no specific direction to the claimed invention. Indeed, this commentary fails to suggest that, contrary to BUCK (as interpreted by the Examiner), primers do not have equivalent sensitivity in the claimed method (as demonstrated in the declarations).

Therefore, this new interpretation does not render obvious the claimed invention, and the rejection of claims 1, 9 and 10 should be reversed.

c. Conclusion

From the foregoing discussion, it is believed to be apparent that the rejections on appeal are improper and should be reversed. Such action is accordingly respectfully requested.

The appeal brief fee of $270 is being paid concurrently herewith online by credit card.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future submissions, to charge any underpayment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

YOUNG & THOMPSON

/Robert A. Madsen/

Robert A. Madsen, Reg. No. 58,543
Attorney for Appellants
209 Madison Street, Suite 500
Alexandria, VA 22314
Telephone (703) 521-2297
Telefax (703) 685-0573
(703) 979-4709

April 1, 2011

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(viii) Claims Appendix

1. A method for determining the presence of colorectal tumors in a human subject, which comprises:

a) extracting DNA from stool samples;

b) amplifying by PCR:

i) p53 gene fragments corresponding to exons 5-8 using the following pairs of primers labeled with fluorescent markers:

1)ctcttcctgcagtactcccctgc(SEQ ID NO:1) and gccccagctgctcaccatcgcta (SEQ ID NO:2),

2)gattgctcttaggtctggcccctc (SEQ ID NO: 3) and ggccactgacaaccacccttaacc(SEQ ID NO: 4),

3)gcgttgtctcctaggttggctctg (SEQ ID NO: 5) and caagtggctcctgacctggagtc (SEQ ID NO: 6),

4)acctgatttccttactgcctctggc(SEQ ID NO: 7) and gtcctgcttgcttacctcgcttagt (SEQ ID NO: 8), and

ii) APC gene fragments using the following pairs of primers labeled with fluorescent markers:

1)aactaccatccagcaacaga (SEQ ID NO: 9) and taatttggcataaggcatag (SEQ ID NO: 10),

2)cagttgaactctggaaggca (SEQ ID NO: 11) and tgacacaaagactggcttac (SEQ ID NO: 12),

3)gatgtaatcagacgacacag(SEQ ID NO: 13) and ggcaatcgaacgactctcaa(SEQ ID NO: 14),

4)cagtgatcttccagatagcc(SEQ ID NO: 15) and aaatggctcatcgaggctca(SEQ ID NO: 16);

c) quantifying the amplified fragments and identifying the amplified fragments as amplicons;

d) calculating the total amount of different amplicons; and

e) comparing the the total amount of amplicons with a reference value determined on the basis of case series comprising healthy subjects and patients affected by colorectal tumors, wherein a total amount of amplicons higher than the reference value is indicative of the presence of colorectal tumors in said human subject.

9. The method according to claim 1, wherein the amplicon quantities are interpolated on a calibration curve obtained from known DNA amounts.

10. The method according to claim 1, wherein the amplicons are quantified with an automatic sequencer/analyser or using fluorimetric, colorimetric, radioactive or spectrophotometric detection systems.

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12. (withdrawn) A kit for determining the presence of colorectal tumors in a human subject, comprising:

oligonucleotides;

labelling agents;

a thermostable DNA polymerase; and

user instructions to carry out the method of claim 1.

(ix) Evidence Appendix

a. A 37 CFR 1.132 Declaration by Daniele Calistri, filed February 16, 2010.

b. Daniele Calistri et al., Detection of Colorectal Cancer by a Quantitative Fluorescence Determination of DNA Amplification in Stool, Neoplasia Vol.6, No. 5, pp. 536-540, 2004, filed February 16, 2010.

c. Loktionov, Cell exfoliation in the human colon: Myth, reality and implications for colorectal cancer screening, Int J Cancer 120:2281-2289(2007) , filed February 16, 2010.

d. A 37 CFR 1.132 Declaration by Daniele Calistri, filed March 14, 2008.

(x) Related Proceedings Appendix

None.